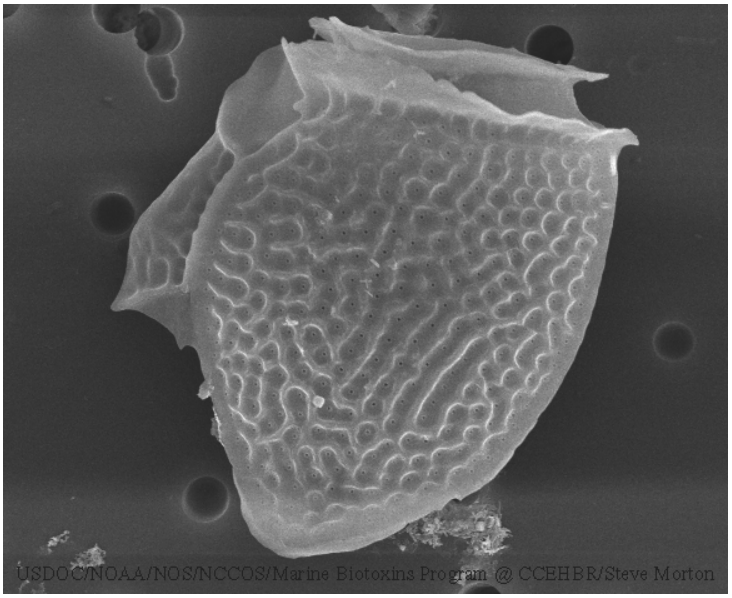
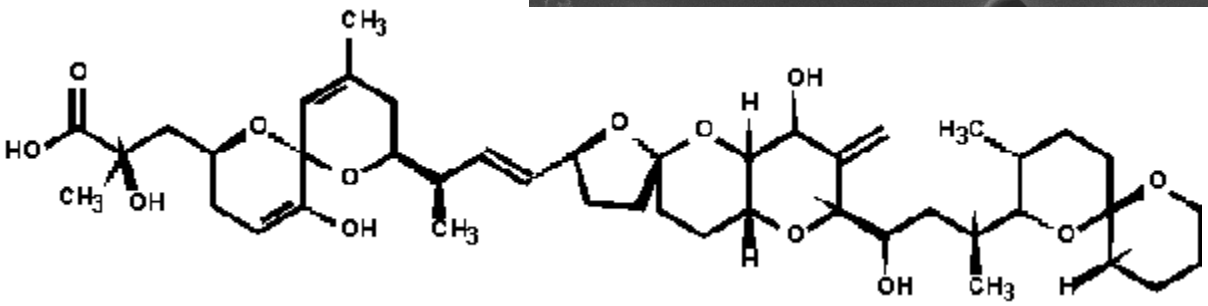
Dinophysis spp.

Description: *Dinophysis* is a toxic dinoflagellate producing toxins associated with DSP (diarrhetic shellfish poisoning). These species are found in North America around the Nova Scotia region of Canada and along the northeast coast of the United States. *P. lima* has also been found in tropical and subtropical regions including Florida, Puerto Rico, and Hawaii.



Toxin Produced: Okadaic Acid and Dinophysis toxin 1 & 2
One of the DSP toxins, Okadaic Acid, is a potent inhibitor of protein phosphatase 2A, and is a first stage tumor promoter.



Syndrome: Diarrhetic Shellfish Poisoning (DSP)
Diarrhetic shellfish poisoning (DSP) produces gastrointestinal effects with an onset of 30 minutes to a few hours, after consumption of contaminated shellfish. Symptoms include diarrhea, nausea, vomiting, and abdominal pain. Long term exposure may promote tumor growth in the digestive system. Full recovery is expected within 3 days regardless of medical treatment.

Distribution: **East Coast**: Northeast United States to southeast Canada coastal waters

Accomplishments: 1996-present

1996: ***Growth Regulation of Toxic Dinoflagellates***
Studies on growth regulation in dinoflagellates have defined the molecular mechanism by which the dinoflagellate cell cycle is phased to the diel cycle. Phasing is accomplished by an inhibitory signal in response to blue light. The blue light receptor in dinoflagellate cells has not yet been identified, but the signaling pathway appears to be dependent on cAMP, a signaling molecule involved in transmitting blue light signals in higher plants. Cell cycle regulatory mechanisms in toxic dinoflagellates will yield useful probes to study the dynamics of harmful algal blooms. Additional investigations carried out this year addressed the role of marine biotoxins in regulating growth dynamics in the ciguatera dinoflagellate community. Ciguatera associated toxins have been identified to elicit allelopathic effects against other co-occurring dinoflagellate species. Results of these studies will provide insight into mechanisms initiating

ciguateric reef conditions. *Contact: Van Dolah*

1997: ***HPLC-Mass Spectrometry of Polyether Toxins***

Highly sensitive and efficient HPLC-MS analyses protocols have been developed for rapid identification and quantification of brevetoxin (PbTx), ciguatoxin (CTX), and okadaic acid (OA), as well as their analogs. This methodology has been optimized to characterize and quantitate these toxins accurately in subnanomolar concentrations. Chromatographic methods as front ends to mass spectrometry have been developed to concurrently allow matrix independent analyses of these toxins. These methods are being implemented to circumvent tedious multiple extractions/pre-purification steps making for more rapid and efficient testing protocols. *Contact: Peter Moeller*

1998: ***Growth Control of Harmful Algal Blooms***

Research on the biochemical pathways that control growth of red tide algae provides a new means to understand the processes that initiate harmful algal blooms and to evaluate measures to control growth of harmful algae. These pathways are amenable to chemical and biological intervention, such as that applied to inhibit growth of terrestrial plants. Current research efforts focus on the Florida red tide dinoflagellate, *Gymnodinium breve*, and the ciguatera associated dinoflagellates. Diel phasing of the cell cycle has been characterized in both laboratory cultures and field populations of the Florida red tide dinoflagellate, and the light dependent cues that couple the cell cycle to the diel cycle have been identified. The molecular regulators of the cell cycle have been shown to be sensitive to inhibition by a drug developed to inhibit growth of cancer cells. Studies on allelochemical interactions within the ciguatera dinoflagellate assemblage have identified a novel growth inhibitory compound produced by *Prorocentrum lima* and active against other dinoflagellates. Liquid chromatography-mass spectrometry has determined that this compound is unrelated to okadaic acid, the biotoxin produced by *P. lima.* Structural characterization and mode of action of this compound are currently are currently being addressed with LC-MS and nmr.
Contact: Van Dolah

1999: ***Evidence of Diarrhetic Shellfish Poison Along the Coast of Maine***

An extensive field survey conducted along the coast of Maine for diarrhetic shellfish poison activity in blue mussels yielded positive results with the protein phosphatase 2A activity. This is consistent with the contamination with okadaic acid or related congeners. Phytoplankton populations from these areas containing contaminated mussels were dominated by *Dinophysis norvegica*, a known toxic species. Two additional known toxic species of *Dinophysis* were also found in low numbers: *Dinophysis acuminata* and *D. rotunda*. However, all plankton samples were negative for phosphatase inhibitory activity. Examination of the epiphytic communities from areas with toxic mussels revealed the first occurrence of the toxic dinoflagellate *Prorocentrum lima* reported from the Northeast. Analysis of epiphytic samples rich in *Prorocentrum lima* were phosphastase inhibitory active. Subsequent analysis of these samples using LC-MS/MS showed the production of dinophysis toxin-1 (DTX-1) by wild populations of *P. lima*. Additional analyses are underway to determine which okadaic acid congener is responsible for the activity found in the blue mussels. This study has provided the first evidence of DSP toxins in U.S. coastal waters.
Contact: Steve Morton

2000: ***Biomonitoring Hawaiian Green Sea Turtles (Eretmochelys Imbricata) For The Presence Of The Tumor Promoter Okadaic Acid***

Fibropapillomas are begin tumors, which in sea turtles are increasing in occurrence. These tumors are fibrous masses of tissue often growing on the eyes, mouth or flippers and on occasions internally in lungs and kidney. These tumors can impede seeing and feeding, are at times fatal and are a threat to recovering species. A joint project with NMFS-Hawaii laboratory was initiated to biomonitor green sea turtles in regions where fibropapillomas are prevalent using the newly developed blood collection card sampling method. The monitoring is conducted for okadaic acid, a dinoflagellate toxin that is a known first stage

tumor promoter. To optimize detection limits, a fluorescent microplate assay has been optimized for use with the rapid blood extraction from the collection cards. Regular sampling and analysis is continuing and positive samples will be related to the disease state of the animals. Blood collection cards have permitted the biomonitoring of toxin in living animals and has greatly increased sampling ease in the field.

Contact: Stacie Dover

Publications:

1. Morphological and biochemical variability of *Prorocentrum lima* (Ehrenberg) Dodge isolated at three sites of Heron Island, Australia. 1995 **J. Phycology** 31:914-922.
2. Survey of toxic epiphytic dinoflagellates from the Belizean barrier reef ecosystem. **Bull. Marine Sci.** 61(3):889-906.
3. Flow cytometric determination of cell cycles and growth rates of *Prorocentrum* spp. 1998 In: **The Physiological Ecology of Harmful Algal Blooms**. pp. 619-648.
4. Okadaic acid production from the marine dinoflagellate *Prorocentrum belizeanum* isolated from the Belizean coral reef ecosystem. **Toxicon** 36 (1):201-206.
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5. Bloom of Gonyaulax polygramma Stein (Dinophyceae) in a coral reef mangrove lagoon, Douglas Cay, Belize. **Bull Mar. Sci.** 63: 639-642.
6. Morphology and toxicology of *Prorocentrum faustiae* sp. nov., a new toxic species of benthic dinoflagellate isolated from Heron Island, Australia. **Botanica Marina**, 41(6):565-570.
7. Evidence for diarrhetic shellfish poisoning along the coast of Maine. **J. Shellfish Res** 18:679-684
8. No evidence for an allelopathic role of okadaic acid among ciguatera associated dinoflagellates. 1999 **J. Phycology** 31:395-400.
9. Determination of okadaic acid from dinoflagellate cells: A comparison between the HPLC method and two monoclonal antibody ELISA test kits. 1996 **Toxicon** 34:947-954.
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10. Okadaic acid production from the marine dinoflagellate, *Prorocentrum belizeanum*, isolated from the Belizean coral reef ecosystem. 1998 **Toxicon** 36(1):201-206.
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11. Evidence for diarrhetic shellfish poisoning along the coast of Maine. **J. Shellfish Res** 18:679-684
12. In vitro detection methods for algal toxins: conceptual approaches and recent developments. **JAOAC International** 84:1617-25
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13. "Shellfish Toxins" Chapter: **Marine and Freshwater Products Handbook**. Technomic Publishing Co., Inc., Lancaster. Pp.727-738.
14. Okadaic acid inhibits a protein phosphatase activity involved in formation of the mitotic spindle of GH4 rat pituitary cells. 1992 **J. of Cellular Physiology** 152:190-198.
    Abstract Available
15. Evidence for diarrhetic shellfish poisoning along the coast of Maine. **J. Shellfish Res** 18:679-684.
16. Health and Ecological impacts of harmful algal blooms: risk assessment needs. **Human and Ecological Risk Assessment 7**: in press.